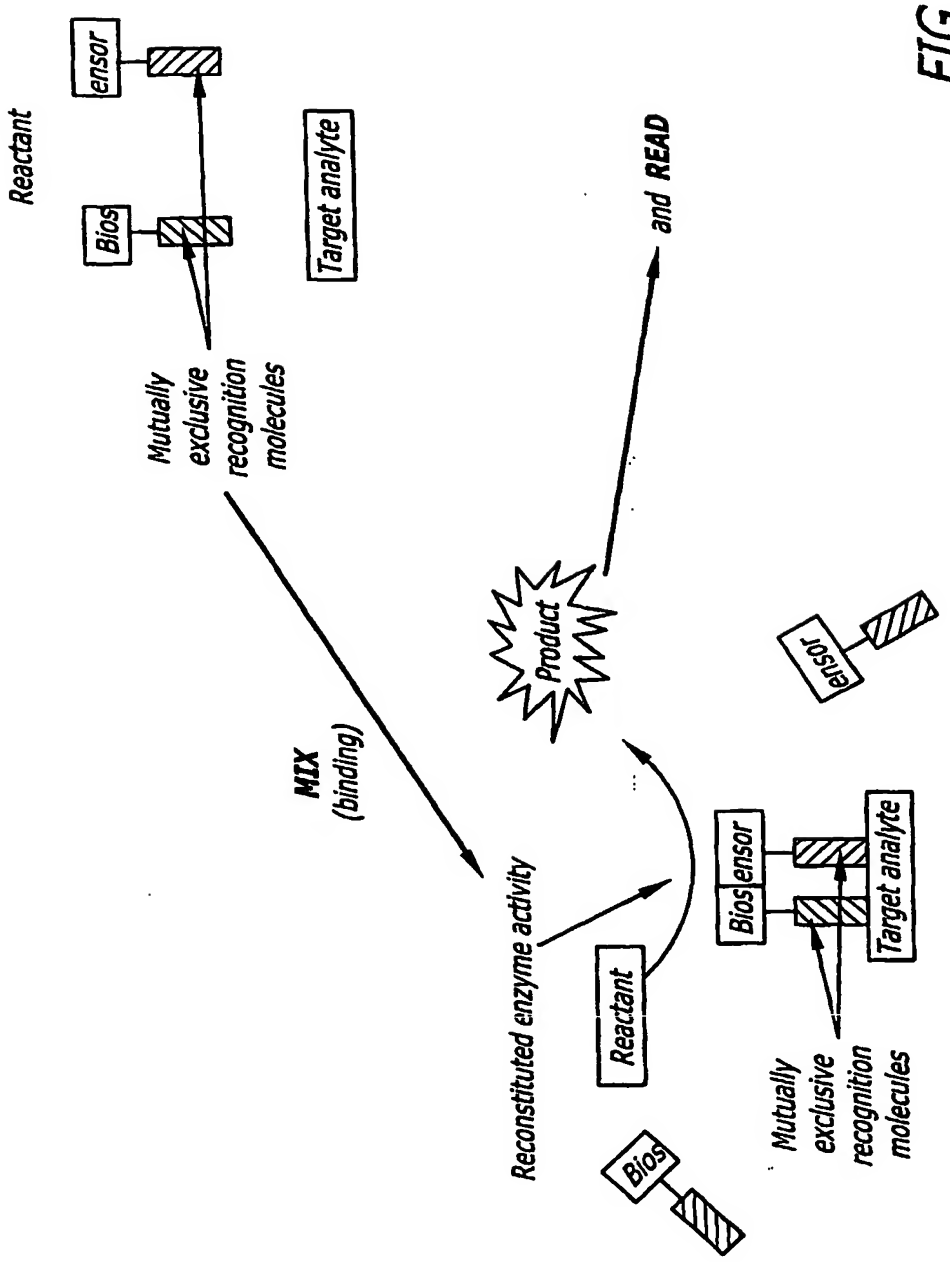

Reactant
ensor
Bios
Target analyte
Mutually exclusive recognition molecules
MIX (binding)
Reconstituted enzyme activity
Reactant
Bios
Product
and READ
ensor
Bios
ensor
Target analyte
Mutually exclusive recognition molecules

FIG. 1

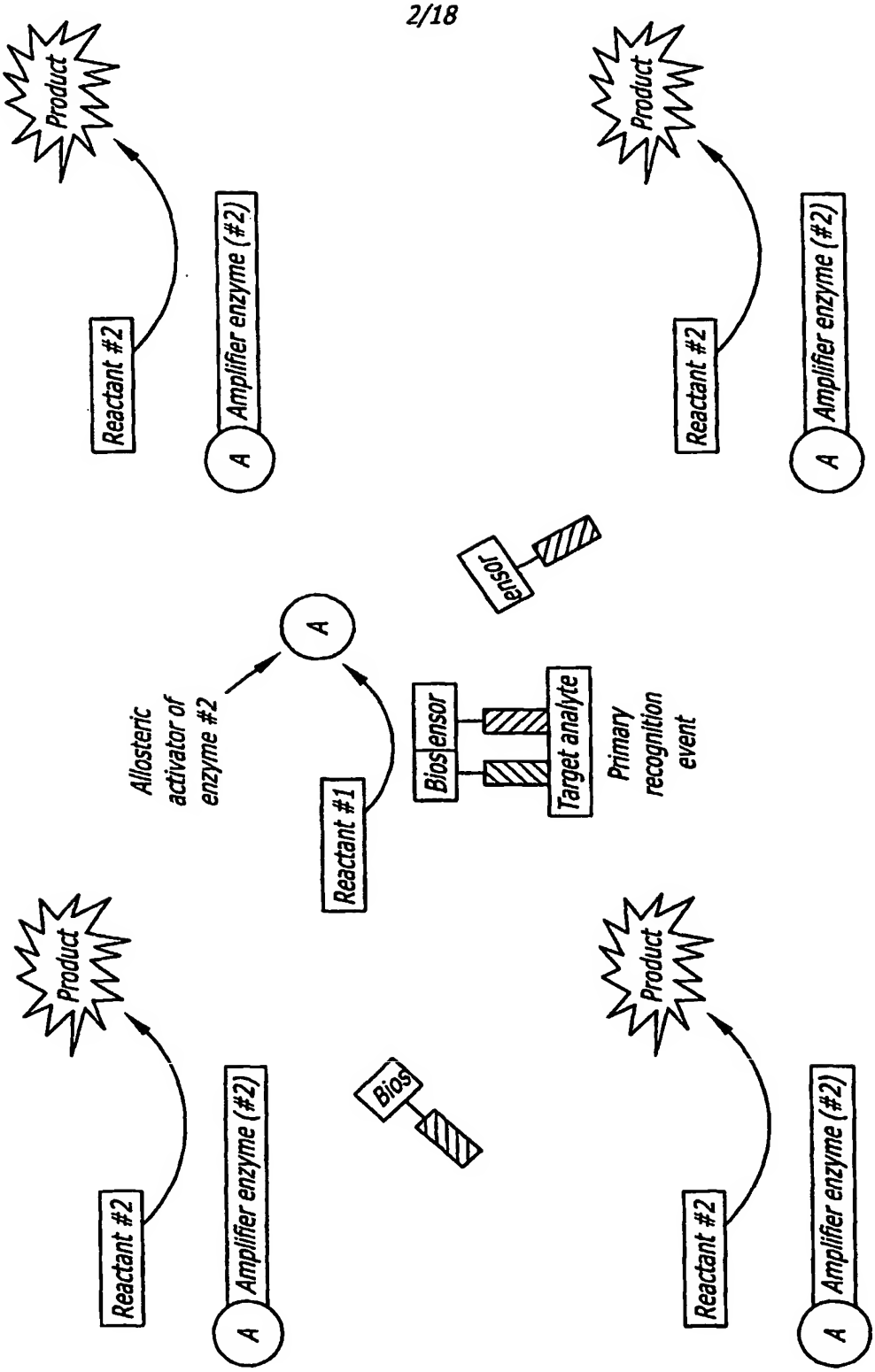

Product
Reactant #2
Amplifier enzyme (#2)
A
Product
Reactant #2
Amplifier enzyme (#2)
A
Sensor
A
Allosteric activator of enzyme #2
Reactant #1
BioSensor
Target analyte
Primary recognition event
Bios
Product
Reactant #2
Amplifier enzyme (#2)
A
Product
Reactant #2
Amplifier enzyme (#2)
A

FIG. 2

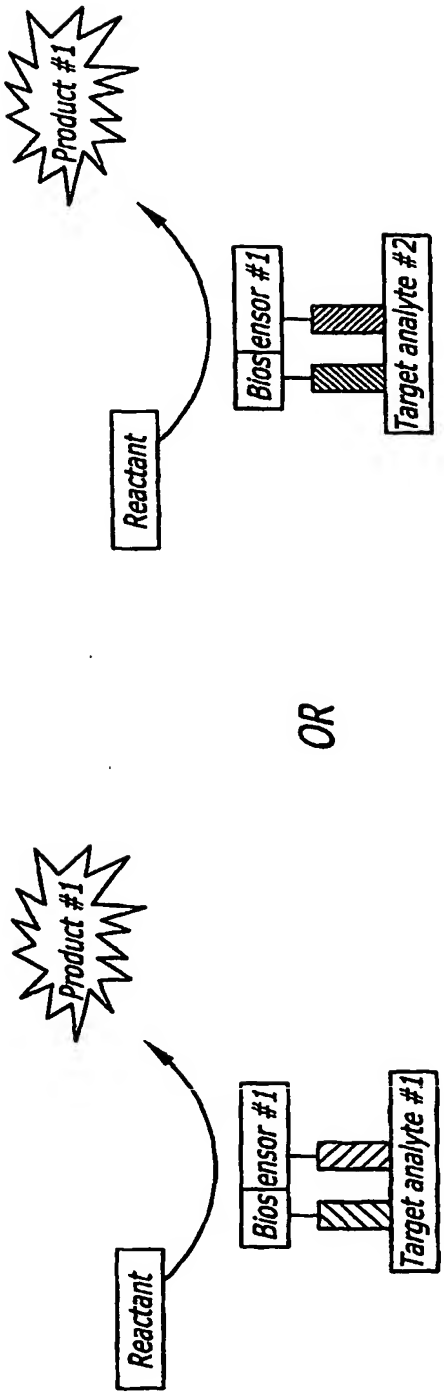

Product #1
Reactant
Biosensor #1
Target analyte #2
OR
Product #1
Reactant
Biosensor #1
Target analyte #1

FIG. 3

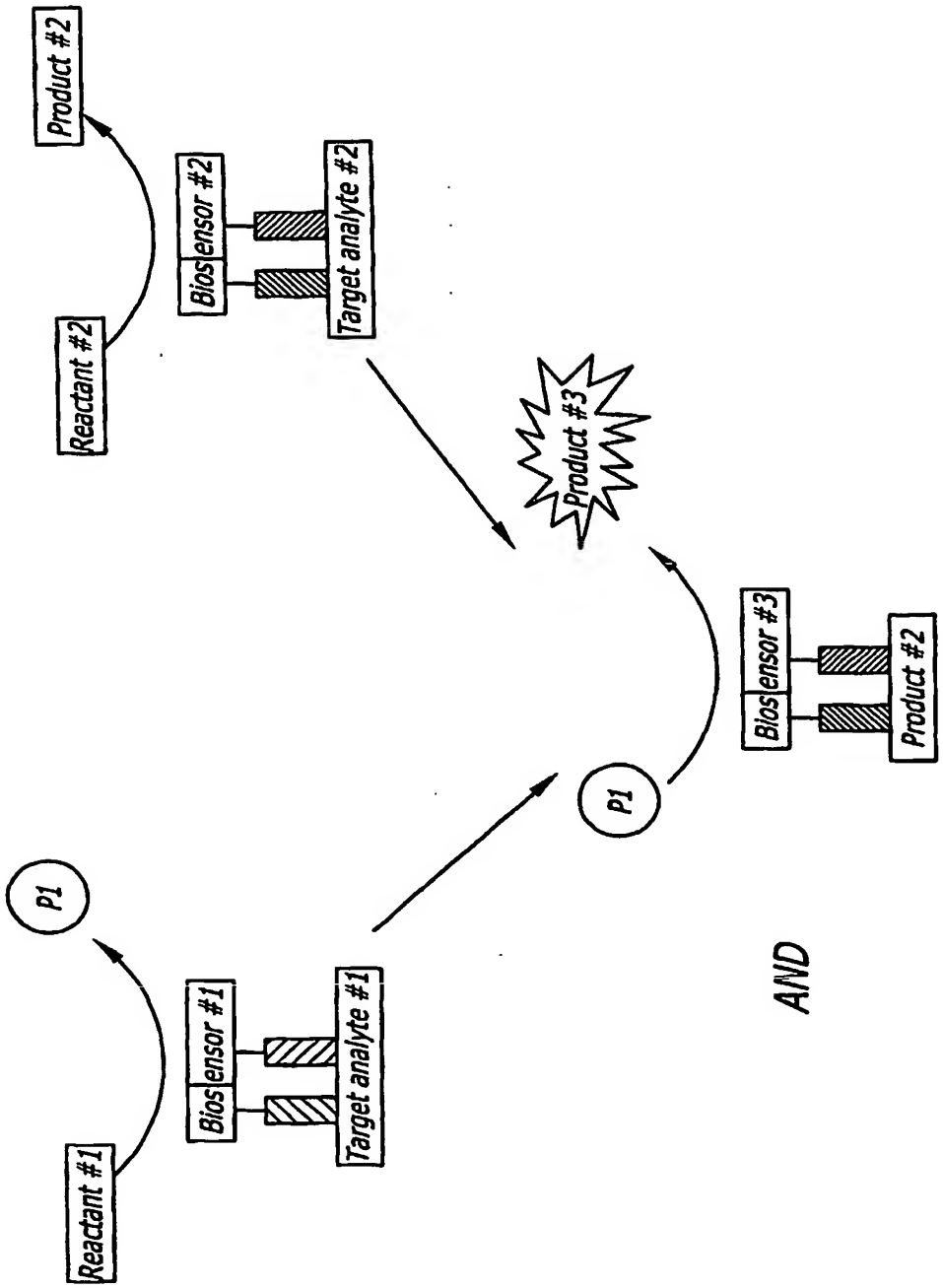

Product #2
Reactant #2
Biosensor #2
Target analyte #2
Product #3
P1
Biosensor #3
Product #2
P1
Reactant #1
Biosensor #1
Target analyte #1
AND

FIG. 4

FIG. 5

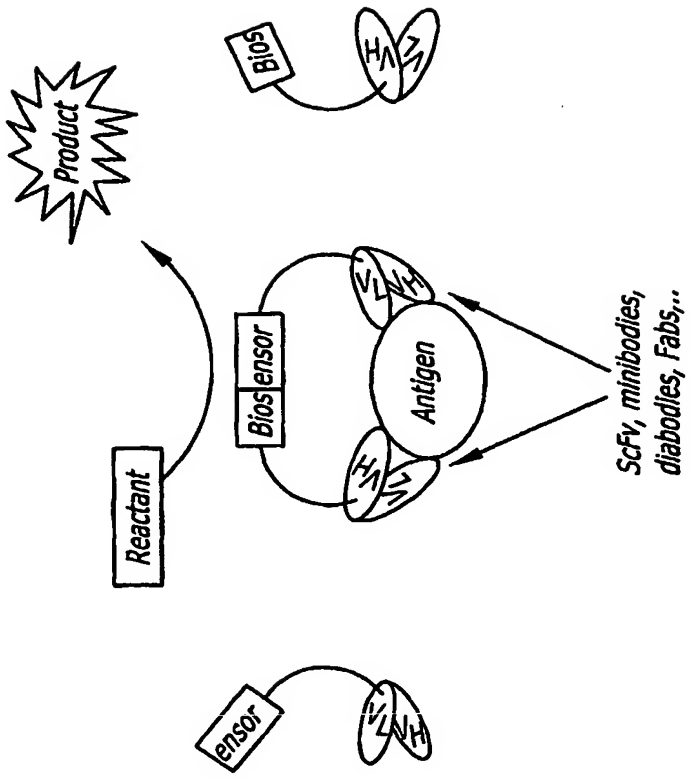

Bios
Product
Bios ensor
Antigen
Reactant
ensor
ScFv, minibodies, diabodies, Fabs,..

FIG. 6

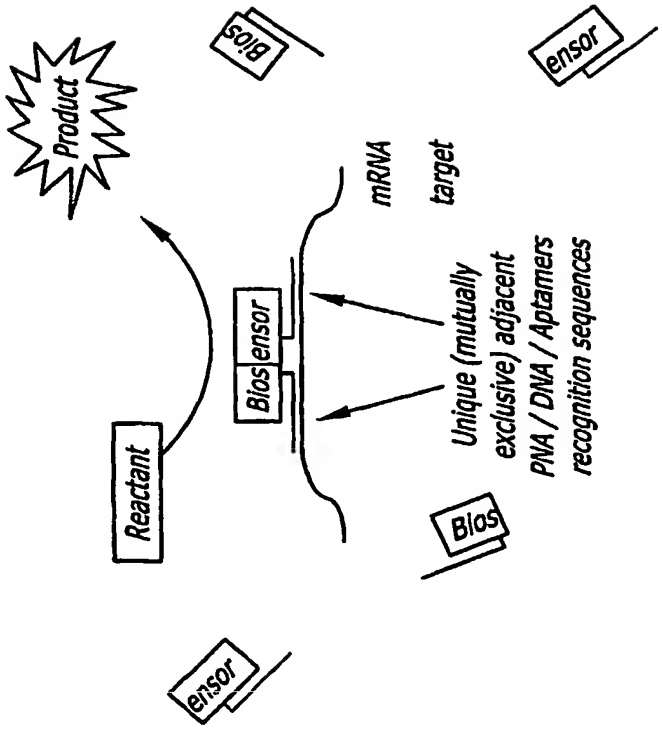

Product
Bios
ensor
mRNA
target
Bios ensor
Unique (mutually exclusive) adjacent PNA / DNA / Aptamers recognition sequences
Reactant
Bios
ensor

FIG. 7

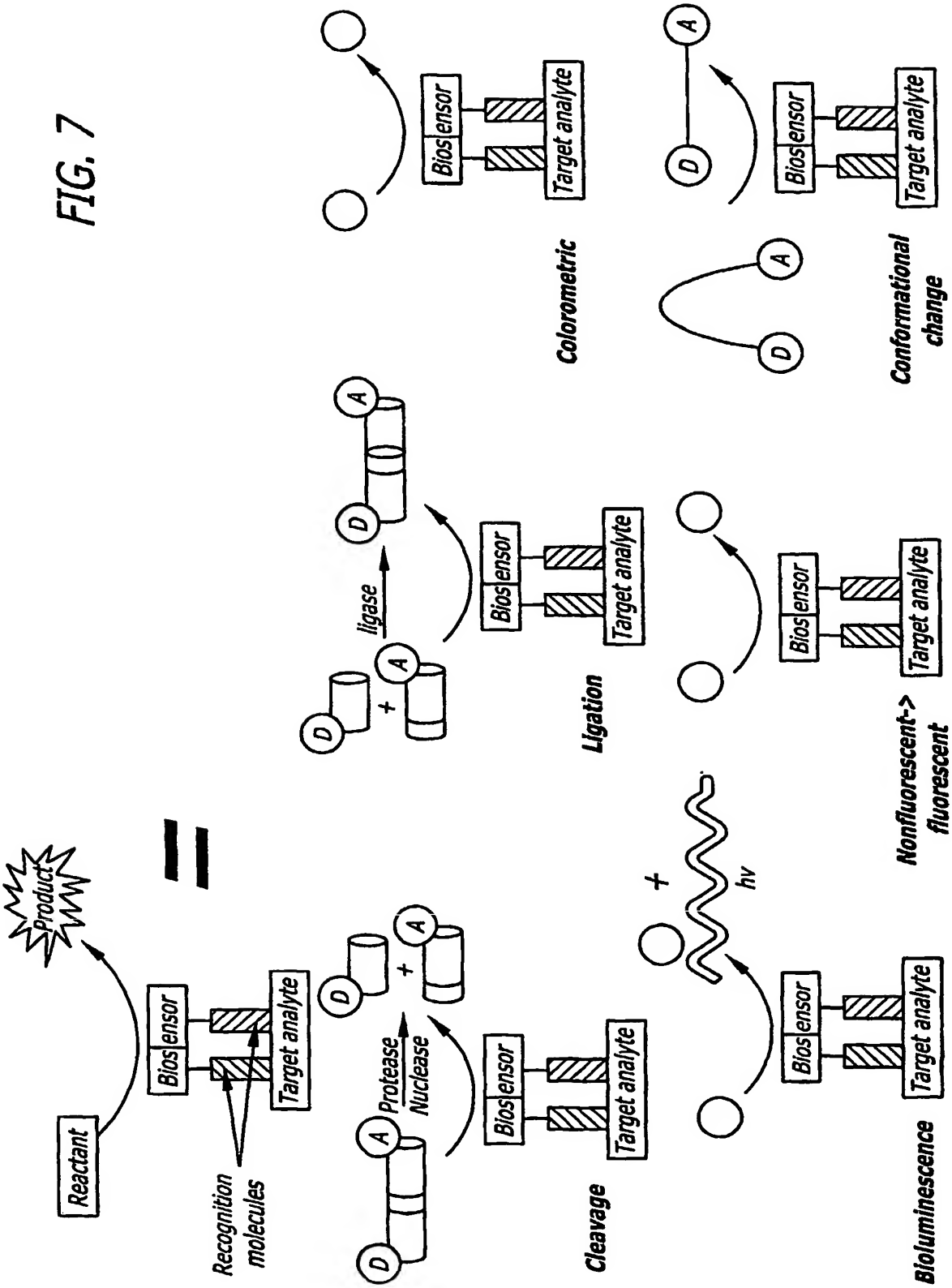

Product
Reactant
Biosensor
Target analyte
Recognition molecules
Protease
Nuclease
Cleavage
ligase
Ligation
Colorometric
Conformational change
hv
Bioluminescence
Nonfluorescent-> fluorescent
A
D

FIG. 8

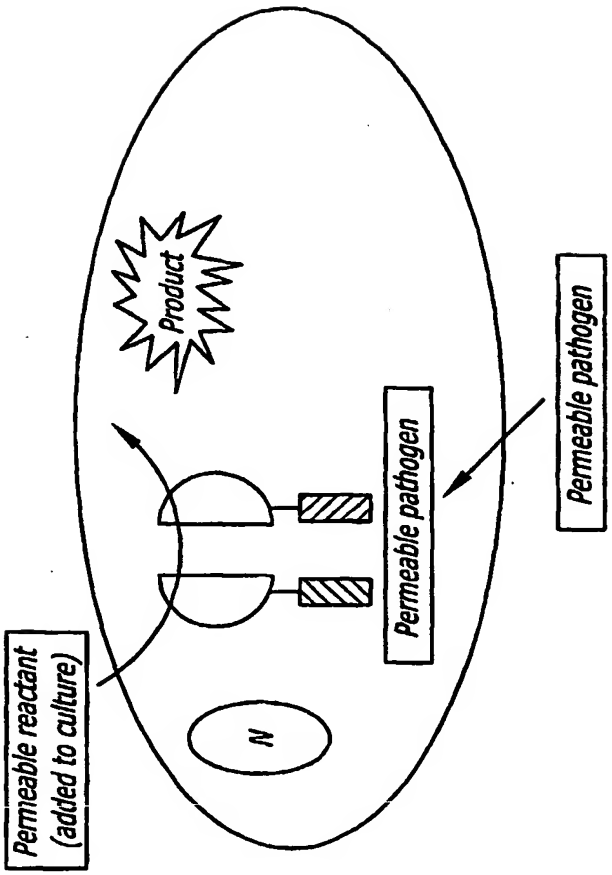
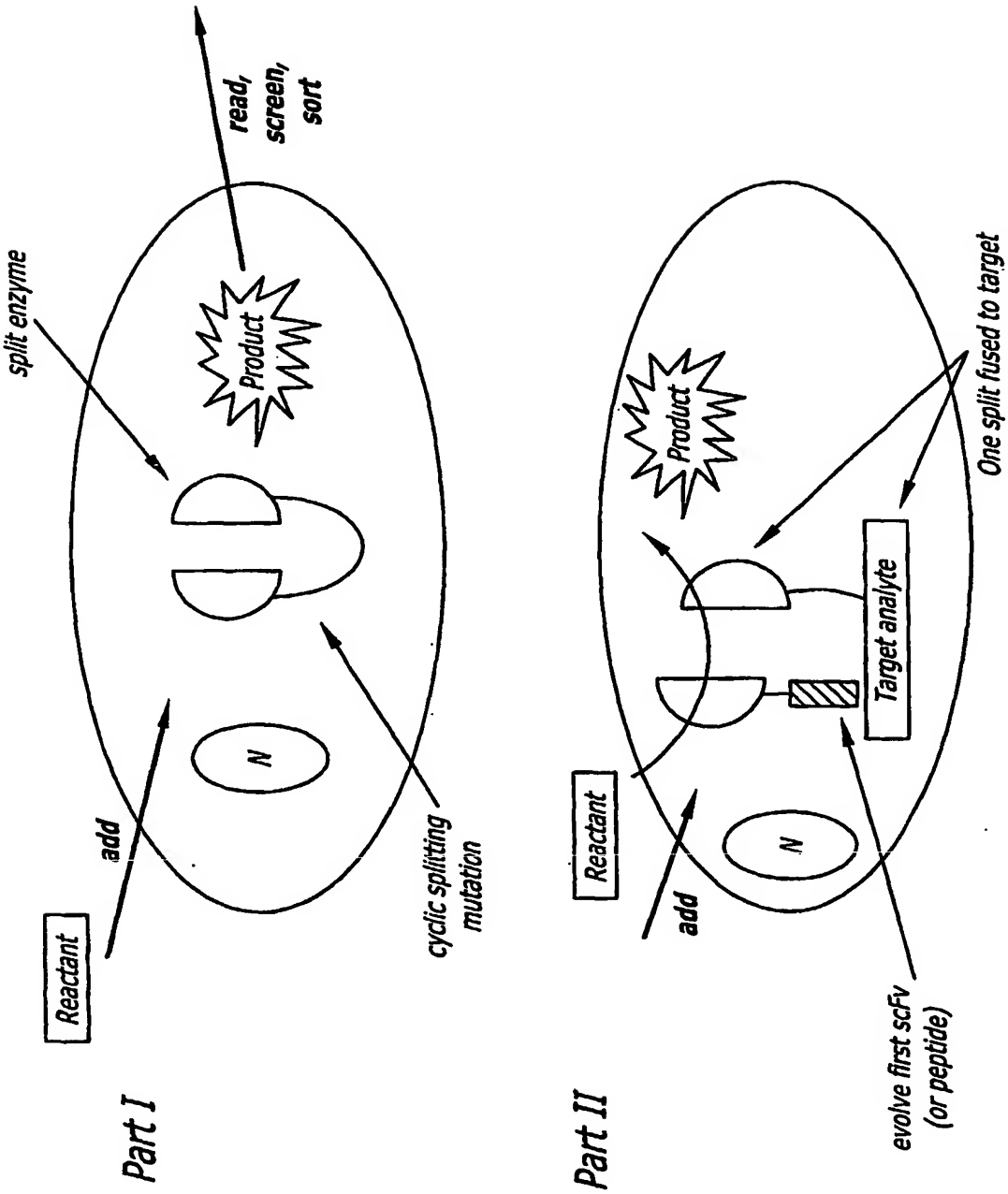

Product
Permeable pathogen
Permeable pathogen
Permeable reactant (added to culture)
N

FIG. 9


Part I
Reactant
add
N
split enzyme
Product
read, screen, sort
cyclic splitting mutation
Part II
Reactant
add
N
Product
Target analyte
One split fused to target
evolve first scFv (or peptide)

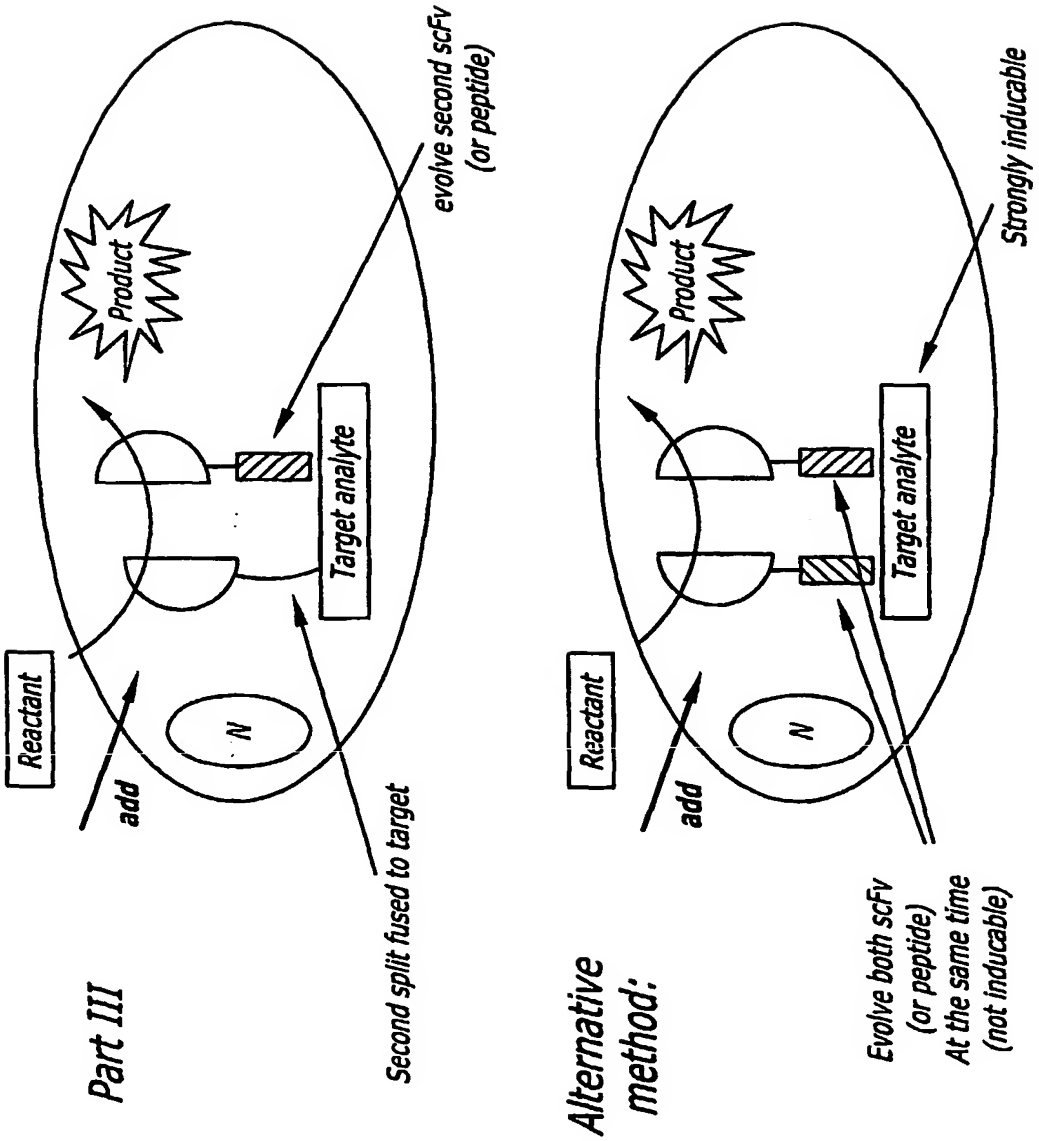
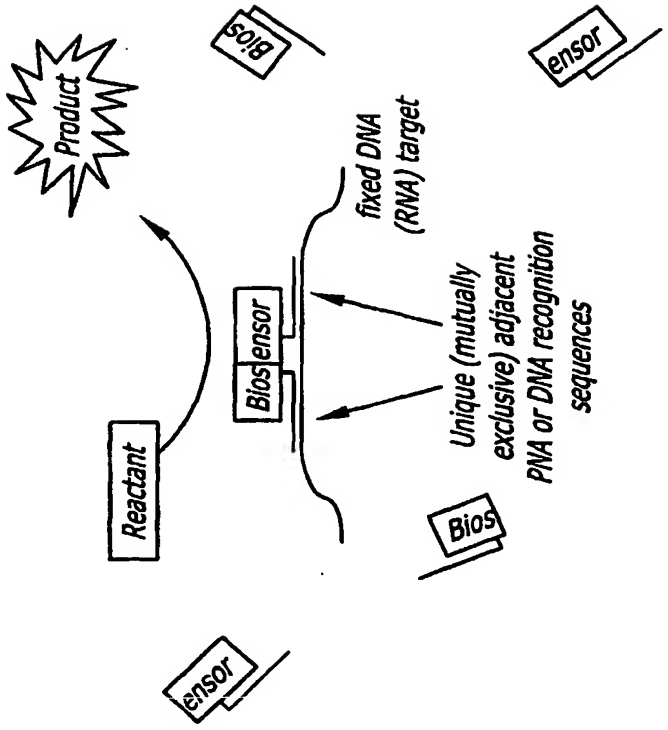

Part III
Reactant
add
N
Product
Target analyte
evolve second scFv
(or peptide)
Second split fused to target
Alternative
method:
Reactant
add
N
Product
Target analyte
Strongly inducable
Evolve both scFv
(or peptide)
At the same time
(not inducable)

FIG. 10

FIG. 11


Product
Bios
ensor
fixed DNA
(RNA) target
Unique (mutually
exclusive) adjacent
PNA or DNA recognition
sequences
Biosensor
Reactant
Bios
ensor

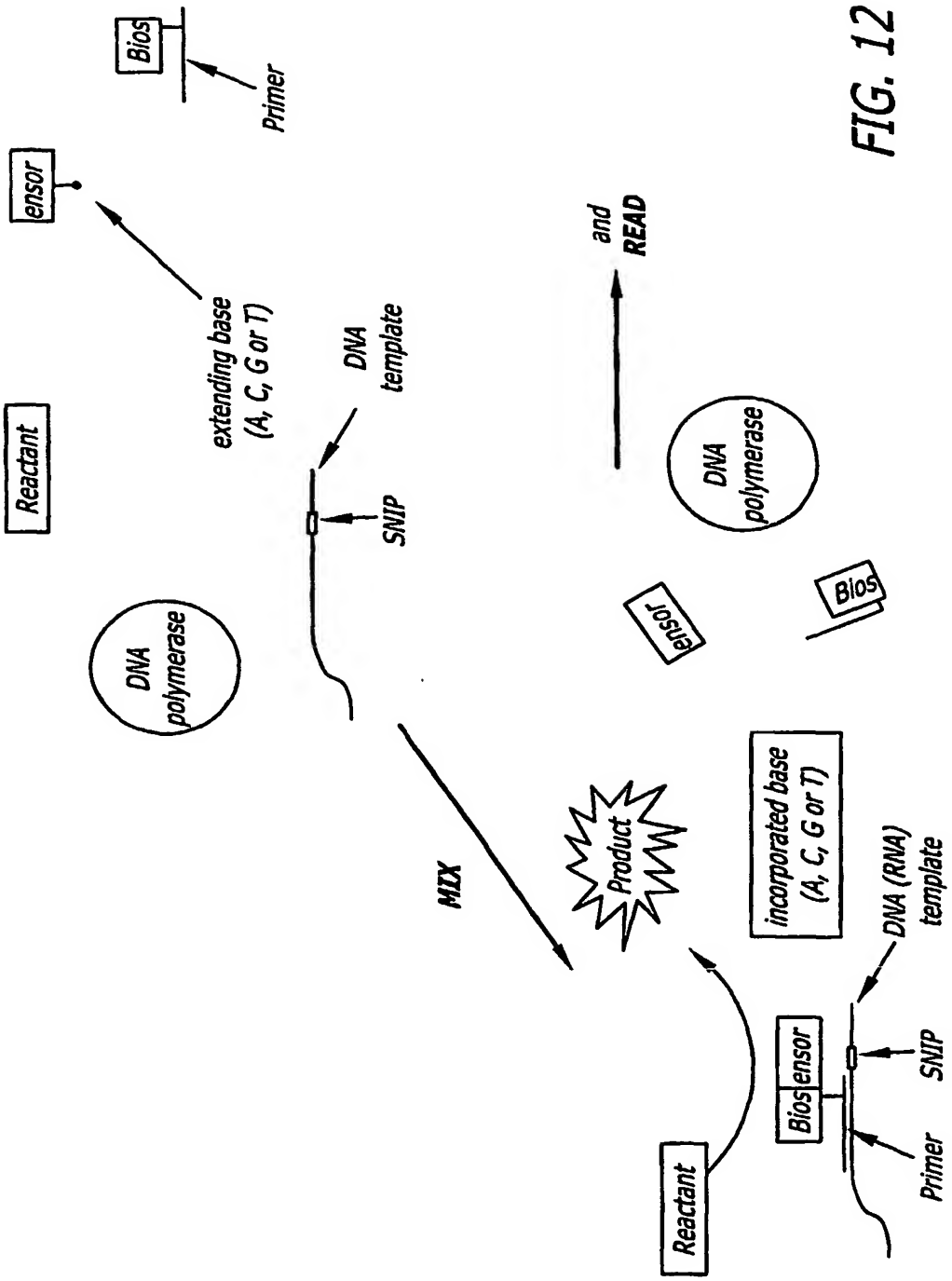

Bios
Primer
ensor
extending base
(A, C, G or T)
Reactant
DNA
template
SNIP
DNA
polymerase
MIX
Product
incorporated base
(A, C, G or T)
and
READ
DNA
polymerase
ensor
Bios
DNA (RNA)
template
Bios ensor
SNIP
Primer
Reactant

FIG. 12

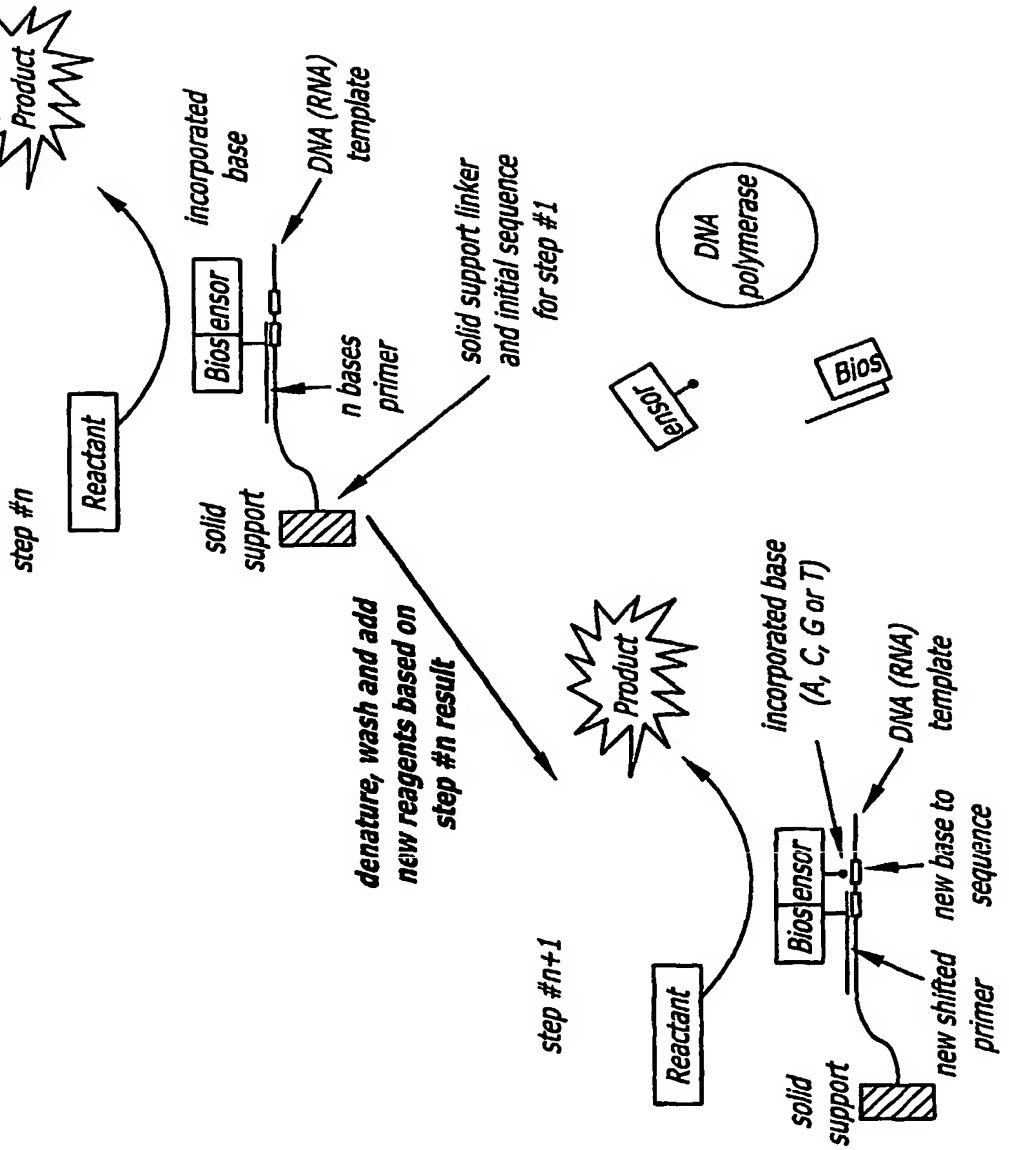
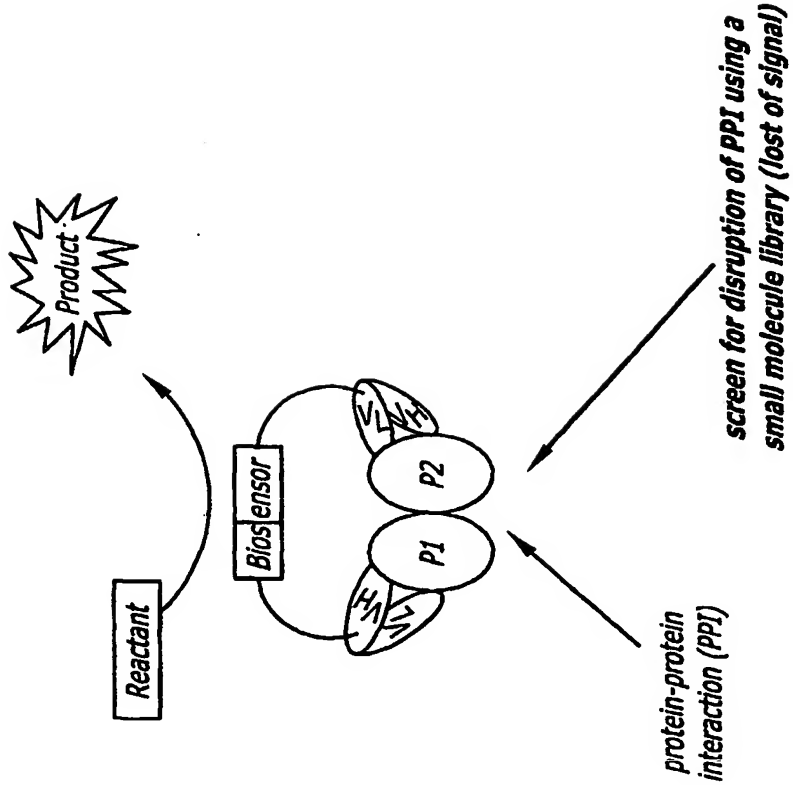

Product
Reactant
step #n
incorporated base
DNA (RNA) template
Biosensor
n bases primer
solid support
solid support linker and initial sequence for step #1
DNA polymerase
Biosensor
denature, wash and add new reagents based on step #n result
Product
Reactant
step #n+1
incorporated base (A, C, G or T)
DNA (RNA) template
Biosensor
new base to sequence
new shifted primer
solid support

FIG. 13

FIG. 14


Product
Reactant
Biosensor
P1
P2
protein-protein interaction (PPI)
screen for disruption of PPI using a small molecule library (lost of signal)

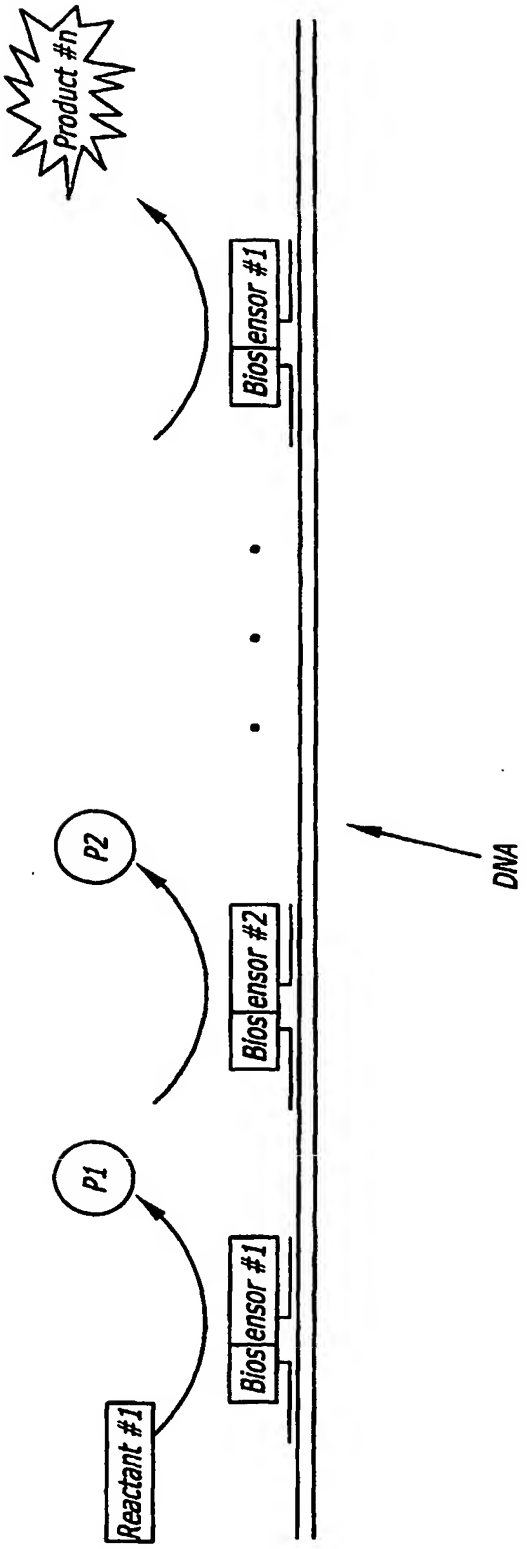

Product #n
Biosensor #1
Biosensor #2
Biosensor #1
P2
P1
Reactant #1
DNA

FIG. 15

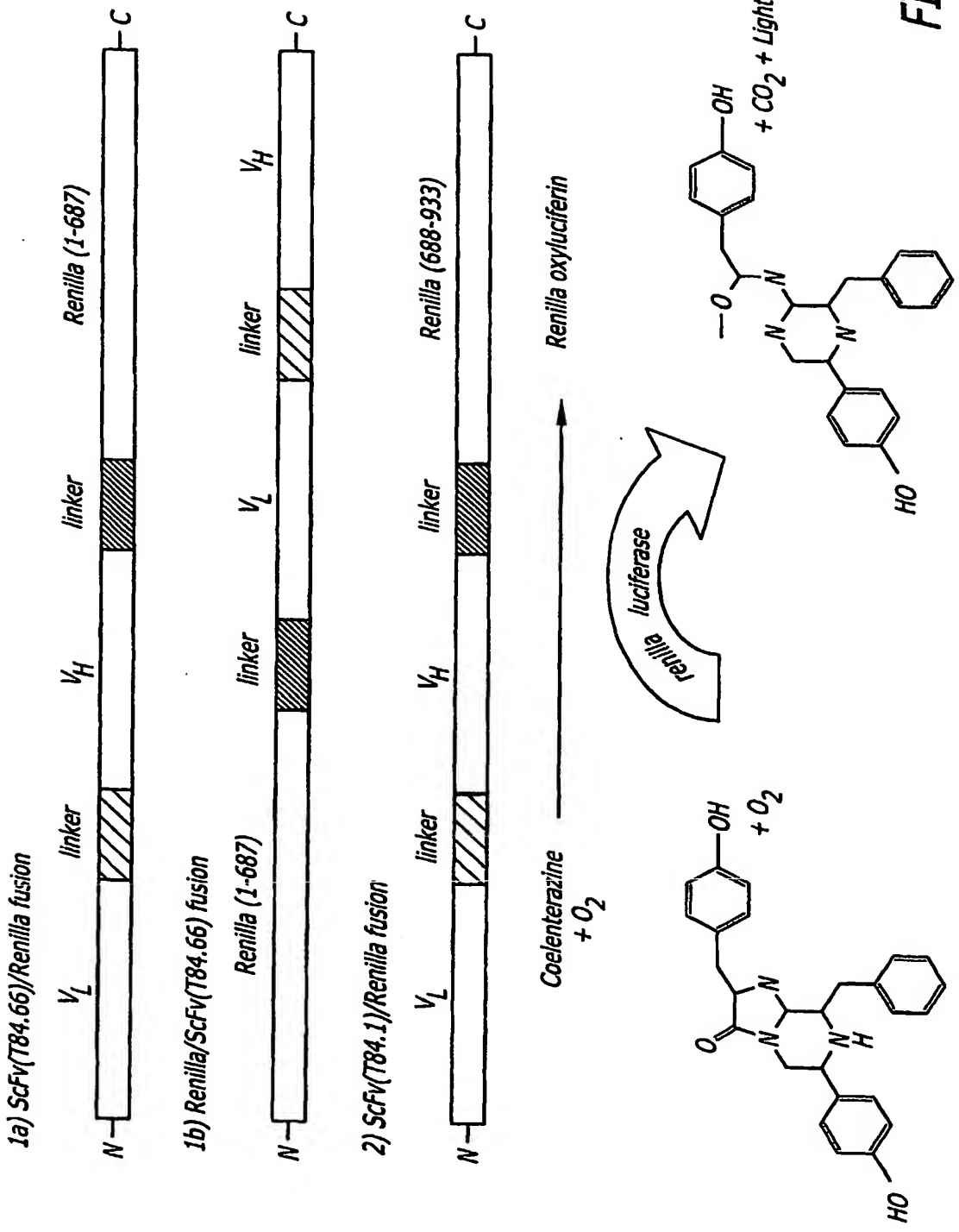

1a) ScFv(T84.66)/Renilla fusion
V_L
linker
V_H
linker
Renilla (1-687)
N
C
1b) Renilla/ScFv(T84.66) fusion
Renilla (1-687)
linker
V_L
linker
V_H
N
C
2) ScFv(T84.1)/Renilla fusion
V_L
linker
V_H
linker
Renilla (688-933)
N
C
Coelenterazine
$+ O_2$
Renilla oxyluciferin
Renilla luciferase
OH
$+ CO_2$ + Light
O
N
N
N
N
HO
OH
$+ O_2$
O
N
N
N
H
HO

FIG. 16

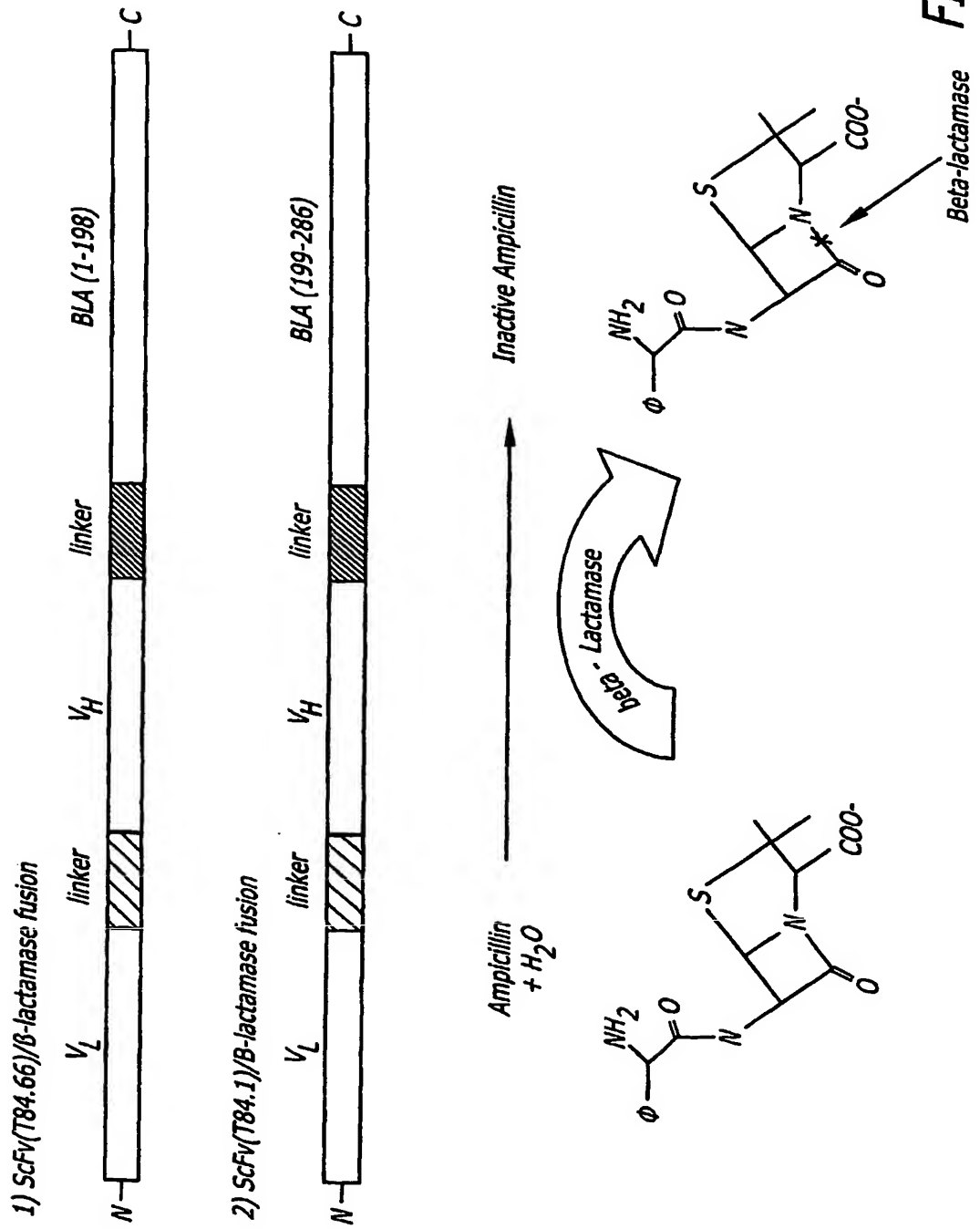

1) ScFv(T84.66)/B-lactamase fusion
VL
linker
VH
linker
BLA (1-198)
C
N
2) ScFv(T84.1)/B-lactamase fusion
VL
linker
VH
linker
BLA (199-286)
C
N
Ampicillin + H2O
Inactive Ampicillin
Beta - Lactamase
Beta-lactamase
NH2
COO-
S
N
O

FIG. 17

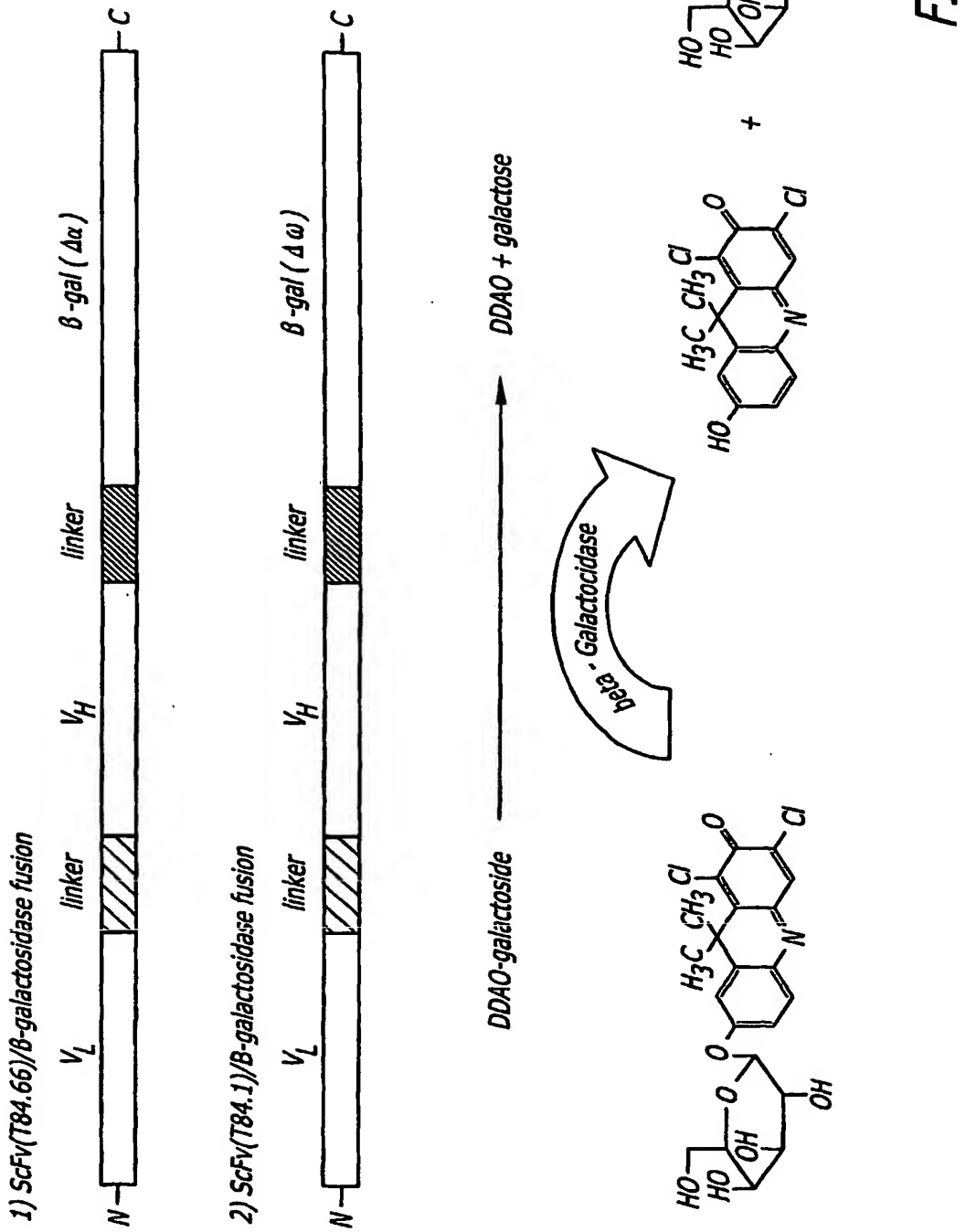

1) ScFv(T84.66)/B-galactosidase fusion
N
C
V_L
linker
V_H
linker
B-gal (Δα)
2) ScFv(T84.1)/B-galactosidase fusion
N
C
V_L
linker
V_H
linker
B-gal (Δω)
DDAO-galactoside
beta - Galactocidase
DDAO + galactose
+

FIG. 18